ENSURING FOOD SAFETY

Panel Manager - Dr. Susan Sumner, Virginia Polytechnic Institute and State University
Program Director - Dr. Etta Saltos

Safety of food products is of paramount importance to the producer, processor, distributor, and consumer. The research program in food safety focuses on research questions involving disease-causing microorganisms, their products, naturally occurring toxicants which contaminate food. The program emphasizes detection, prevention, and control. Projects may focus either on pre- or post-harvest/slaughter origin of the microbial agent or toxicant. Additionally, the program supports research on the identification of obstacles to adopting safe food habits, with particular emphasis on factors affecting consumer attitudes and behavior, as well as the development of intervention strategies to improve food safety habits.

2000-02590 Novel Molecular Approaches to Eradicating E. coli O157 From the Bovine GI Tract
Foster, J.W.
University of South Alabama; Department of Microbiology and Immunology; Mobile, AL 36688
Grant 01-35201-09955; $230,810; 3 Years

Enterohemorrhagic *E. coli* (EHEC) cause a variety of gastrointestinal infections. EHEC serotype O157:H7 has emerged as an important foodborne pathogen that threatens many aspects of the food industry. A crucial component of O157:H7 pathogenesis and epidemiology is its ability to survive environmental stresses imposed by the infected host. The successful pathogen survives this assault through the induction of microbial stress response systems. The investigators have identified two EHEC stress response systems essential for this organism to survive in the bovine gastrointestinal tract, an important reservoir of O157:H7. This project will test strategies designed to corrupt these stress response systems, thereby rendering O157 unable to survive in the gastrointestinal tract. (Strategy 1) A natural *E. coli* peptide was found that dramatically inhibits the activity of σ^S, an important stress response sigma factor required to transcribe numerous stress response genes. The smallest peptide retaining anti-σ^S activity will be determined and its activity optimized for use as an antimicrobial agent. (Strategy 2) Several O157:H7-specific bacteriophage have been identified and will be used to design an antimicrobial phage Acocktail@ that will be tested for its ability to kill O157 In the calf shedding model. In addition to phage that can kill O157, other phage may be useful as O157 Asmart bombs@ designed to deliver genes encoding antimicrobial peptides directly to O157. Although this project specifically addresses the problem of O157 in the farm environment, its success will have a broad impact on the design and implementation of antimicrobial therapies for many infectious agents.

2000-02589 Effect of Phagosome Activities on *Campylobacter jejuni* Pathogenesis
Joens, L.A.
University of Arizona; Department of Veterinary Science and Microbiology; Tucson, AZ 85721
Grant 01-35201-09948; $250,000; 3 Years

Campylobacter jejuni is considered responsible for 3 million cases of acute gastroenteritis per year, making it the single most common cause of bacterial gastroenteritis in the U.S. Few factors associated with *C. jejuni* virulence have been identified. Progress in this important research area has been hampered by the lack of genetic tools (transposons, transduction systems) to examine or identify virulence factors. To date, roughly 90 *C. jejuni* genes have been characterized, only six of which are related to virulence. Therefore, the construction of the katA mutant and the demonstration that catalase production is important in the survival of the organism in phagocytic cells is a major step toward understanding mechanisms this pathogen uses to successfully interact with the host and cause disease. The investigators will determine what effect phagosome acidity has on the survival of *C. jejuni*. The maturity

of the *C. jejuni*-phagosome will be determined. Inhibitors to the formation of the respiratory burst and precursors for nitrous oxide production will be used to abrogate the bactericidal activity of the phagosome. Finally, using the newborn pig model, the investigators will examine the pathogenicity of both the sodB and katA mutant.

2000-02446 Immunoelectrochemical/Optical Biosensor with a Capillary Bioseparator/Bioreactor for Rapid Detection of Pathogens in Poultry and Meat Products

Li, Y.; Liu, Y.
University of Arkansas; Department of Poultry Science; Fayetteville, AR 72701
Grant 01-35201-10056; $130,000; 1.5 Years

The overall goal is to develop a biosensor system for rapid detection of several major pathogens in poultry and meat products. Three phases will be followed to approach the goal: (1) design and fabricate a capillary immuno-separator/bioreactor for bacterial separation and enzymatic amplification; (2) develop a flow-injection biosensor system based on the immuno-separator/bioreactor and electrochemical/optical transducers; and (3) evaluate the biosensor system using processed raw and cooked poultry and meat products. In Phase I, to be funded first, an immuno-separator/bioreactor will be designed and constructed using a capillary column with immobilized antibodies. A layer of active membrane will be coated on the inner wall of the capillary for bonding of antibodies. By injecting samples and then enzyme-labeled antibodies, antibody-pathogen-enzyme labeled antibody conjugates will be formed in the capillary. Substrates will be injected to generate the signals required for electrochemical/optical measurements. The conditions of antibody immobilization, bacterial separation, enzymatic reaction and amplification will be optimized to maximize the capture efficacy and biological signals and minimize noises. Major pathogens found in raw and cooked poultry and meat products, including *Salmonella typhimurium*, *Listeria monocytogenes*, *E. coli* and *Campylobacter jejuni*, will be tested. This proposed research will provide the poultry and meat industry with new technology for rapid detection of pathogens on site or on line. This will help the industry enhance HACCP programs, minimize product recalls and clear international trade barriers due to microbial contamination. Consequently, this will help the whole society ensure food safety, and reduce foodborne diseases and related medical cost.

2000-02635 Factors Affecting Colonization of Plants by Human Pathogenic Bacteria

Lindow, S.E.; Suslow, T.
University of California, Berkeley; Department of Plant and Microbial Biology; Berkeley, CA 94720-3102
Grant 01-35201-10058; $275,000; 2 Years

The surfaces of plants are normally colonized by large numbers of bacteria, called epiphytes, which include plant pathogens, other saprophytic and beneficial bacteria as well as human pathogenic bacteria such as *E. coli* and *Salmonella.* Preliminary studies have shown that while *E. coli* and *Salmonella* strains can grow on moist plants, they are hypersensitive to the stress of dry leaf surfaces compared to other bacteria found on plants, indicating that stress tolerance is a determining factor in their epiphytic colonization. We will determine the differences among successful plant colonists and human pathogens in such intrinsic stress tolerance by measuring their growth and survival after inoculation onto plants under a variety of controlled conditions as well as in the field. This information will enable the extensive background information on other leaf surface colonists to be extrapolated to predict the behavior of human pathogens on plants and to devise better methods to avoid or eradicate human pathogens on plants. While epiphytes can occur as solitary cells, the majority of the population often occurs in relatively large aggregates which can contribute to the survival of stresses on leaves and successful immigration to a leaf. We will determine the extent to which the survival of human pathogens on leaves is dependent on the numbers of indigenous bacteria present on a leaf and, at small scales, will study the role of bacterial aggregates in survival and proliferation of such strains using microscopic techniques to quantify and evaluate the viability of bacteria on leaves.

2000-02539 DNA Adenine Methylase Mutants of *S. typhimurium* as Modified Live Vaccines in

Calves

Mahan, M. J.
University of California, Santa Barbara; Dept. of Molecular, Cellular, and Developmental Biology; Santa Barbara, CA 93106

Grant 01-35201-10188; $310,000; 3 Years

Salmonella is the most commonly isolated infectious enteric bacterial pathogen of dairy cattle and the most common disease associated with human consumption of beef and dairy products. In recent years there has been a rise in the incidence and severity of human cases of salmonellosis, in part due to the emergence of the antimicrobial resistant *S. typhimurium* in cattle populations. On large commercial dairy farms it is very common for cattle to be exposed to many Salmonella strains and for calves to become infected shortly after birth. Under these conditions it would be desirable to have a Salmonella vaccine capable of stimulating immunity to many Salmonella strains. The overall goal of this proposal is to test the hypothesis that *S. typhimurium* lacking the DNA adenine methylase (Dam) are effective live vaccines against Salmonella infection of cattle. This proposal is based on our recent discovery that *S. typhimurium* containing mutations in the Dam gene are totally avirulent yet confer full protection against murine typhoid fever when used as live vaccines. We propose to determine whether Dam-derivatives of Salmonella are attenuated for virulence in calves and whether they can serve as live vaccines that elicit immunity to many Salmonella strains. The proposed vaccines will have a profound influence on reducing salmonellosis in cattle and on improving the safety of beef and dairy products. Moreover, these vaccines may have utility in other livestock species, which would provide a means of controlling Salmonella in livestock production systems and enhancing the safety of the food supply.

2000-02490 Mortality Kinetics of Bacterial Populations Exposed to High Pressure

Hoover, D.G.; Daniels, W.B.
University of Delaware; Department of Animal & Food Sciences and Department of Physics & Astronomy; Newark, DE 19717-1303

Grant 01-35201-09947; $230,000; 3 Years

The goal of this project is to collect and analyze data from the high hydrostatic pressure processing (HPP) of microbial pathogens and surrogate organisms in foods using a multiple-vessel pressure unit designed for measurement of microbial destruction kinetics. This research project will provide information germane to appropriate processing parameters which can be applied to assure the microbiological safety of foods preserved using HPP. Modeling the information will provide a summary of the experimental data for use in future commercial applications. This will increase in importance as HPP becomes more readily accepted by the food industry. U.S. and international consumers are demanding minimally processed foods that resemble raw or fresh-like products in sensory quality (*e.g.*, flavor, texture and appearance) and nutrient content as compared to their highly heat-processed counterparts, while retaining similar levels of safety and convenience. This project will specifically address the nonlinear nature of HPP-derived semilogarithmic survival curves. Since a logarithmic (first-order) rate of death is normally assumed in the predictive microbiology of HPP, there is a food safety concern associated with extrapolation of the process end-point beyond the linear region. This practice carries the danger of underestimating HPP-resistant organisms remaining in the processed food. Since limited experience and history exist on use of HPP in the production of large quantities of food, the reliance on first-order kinetics to project the level of risk and process effectiveness warrants examination to ensure the safety of HPP foods.

2000-02512 Microbiological Safety of Citrus Fruit for Juice Processing

Goodrich, R.M.; Parish, M.E.
University of Florida; Citrus Research & Education Center; Lake Alfred, FL 33850

Grant 01-35201-10059; $200,000; 2 Years

Fruit products such as citrus juices have traditionally been considered "low-risk" with respect to foodborne

illness. High levels of acidity, characteristic of citrus juices, have not typically supported the growth of human pathogens. Furthermore, because almost all citrus juices undergo significant food processing steps such as concentration, freezing, aseptic packaging, and/or pasteurization, the overall food safety risk to Americans consuming citrus juices has been quite minimal throughout the 50-year history of mass citrus juice marketing. However, in the last 5 years, a number of high profile cases of foodborne illness have been linked to the consumption of fruit juices, particularly apple and orange juices. In most of these cases, the juice was consumed in a fresh (non-pasteurized) form. Fresh juice is preferred by some consumers who view it as a more natural product with superior flavor characteristics. However, it is clear that fresh juice presents a potential food safety risk, and the U.S. Food and Drug Administration (FDA), who is charged with ensuring the safety of fresh juices, has little actual data available to assess the extent of these risks. Successful completion of this project, which will extensively evaluate the microbial contamination of oranges entering a juice extraction facility over the course of 2 fruit seasons, will provide data to both fresh and processed juice producers regarding the potential for further foodborne illness associated with citrus juice. This research will directly impact an industry with almost $10 billion in consumer sales, and provide information from which science-based food safety regulations can be formed.

2000-02630 Phase variation and expression of capsular polysaccharide in *Vibrio vulnificus*
Wright, A.C.
University of Florida; Department of Food Science and Human Nutrition; Gainesville, FL 32611
New Investigator Award; Grant 01-35201-09954; $260,000; 3 Years

Vibrio vulnificus is a natural bacterial inhabitant of bays and estuaries along the entire U.S. coastline. It causes devastating disease in humans and continues to be the leading cause of mortalities related to seafood consumption. Disease is primarily associated with eating raw oysters or with exposure of wounds to seawater. Aggressive attempts to increase public awareness have not been successful in reducing the number of cases, and increased dependence on foreign seafood markets and aquaculture may exacerbate the problem. The underlying disease mechanisms are still elusive; however, several laboratories have demonstrated that most, if not all, virulent strains produce a polysaccharide capsule. Interestingly, when grown under laboratory conditions, about 1 in every 1000 encapsulated cells spontaneously becomes capsule deficient and is no longer able to cause disease. This process is called phase variation and is a common theme for a number of bacterial species. The process is reversible, and deficient strains can recover capsular polysaccharide (CPS) expression. Phase variation may be driven by selective pressure from environmental factors such as starvation or stress. The genetic basis for phase variation in *V. vulnificus* is unknown. The investigators' previous work described factors that regulate CPS expression and identified a genetic locus for *V. vulnificus* CPS. Phase variation correlated with rearrangements at this locus. The proposed research will investigate the genetic basis for phase variation, and its influence on survival in the oyster. These studies should elucidate important disease determinants, increase understanding of the relationship of *V. vulnificus* to oysters, and perhaps provide future technologies with applications to seafood and aquaculture industries.

2000-02515 Persistent Colonization by *E. coli* O157:H7 in Ruminants
Cornick, N.A.
Iowa State University; Veterinary Medical Research Institute; Ames, IA 50011
New Investigator Award; Grant 01-35201-10057; $185,000; 3 Years

Escherichia coli O157:H7 and other Shiga toxin-producing E. coli (STEC) cause acute gastrointestinal illness in people. The majority of human disease is associated with the consumption of contaminated foods, many of which originate from cattle. The principal problem appears to be that STEC become established in the intestinal tract of mature cattle or sheep, which then serve as a reservoir. The long-term goal of the proposed research is to eliminate cattle and sheep as a reservoir of STEC. The central hypothesis is that virulence factors produced by STEC confer an advantage to the organism that allows for the development of an asymptomatic carrier-shedder state. In preliminary

studies, the investigator has developed a sheep model for the carrier-shedder state of *E. coli* O157:H7. This model will be used to complete two objectives to test the hypothesis: 1) Determine if intimin (a bacterial protein required for one type of attachment in the intestine) is required for *E. coli* O157:H7 to establish a persistent population in mature sheep. 2) Determine if Shiga toxin increases the population of *E. coli* O157:H7 in mature sheep. This research will benefit US agriculture by determining if either intimin or Shiga toxin should be further investigated as a potential vaccine candidate to eliminate *E. coli* O157:H7 from cattle and sheep.

2000-02908 Whole Genome Scan for Host-Adapted *Salmonella* Pathogenicity Genes in Swine

Lichtensteiger, C.A.; Vimr, E.R.
University of Illinois, Urbana-Champaign; Department of Veterinary Pathobiology; Urbana, IL 61802
Grant 01-35201-09949; $250,611; 3 Years

Salmonella is a top foodborne pathogen of concern in the pork industry. Pork-based salmonellosis starts with carcass contamination at the slaughter house with feces from infected pigs (non-ill carrier pigs). Raising *Salmonella*-free animals is the ultimate means to prevent foodborne salmonellosis. The mechanism of *Salmonella* infection (colonization) varies with animal host and bacterial species. To gain basic information on colonization mechanisms of *Salmonella* in pigs, we propose a functional genomics study of *S. typhimurium* and *S. choleraesuis.* In our study we will generate signature tagged mutants of *Salmonella* and screen the pools of mutants for loss of ability to colonize pigs. The mutants will have a random gene tagged; this will inactivate the gene and provide a unique nuclear tag for mutant identification. Mutants that fail to colonize will be confirmed attenuated by a mixed challenge infection with wild type *Salmonella*. The signature tag will also allow cloning, sequencing, characterizing the gene that was inactivated. Some of the colonization genes will have functional homologues in the DNA database; while others will have structural homologues previously unknown to be involved in colonization or be novel genes not previously known. The colonization genes or their protein products are targets for future applied studies to develop means to raise *Salmonella*-free pigs. These means include novel vaccines that prevent colonization or competitive exclusion cultures by non-pathogenic bacteria that inhibit colonization by pathogenic bacteria.

2000-02587 Genetic Determinants of *Salmonella* in Chickens and Mice

Maloy, S.
University of Illinois, Urbana-Champaign; Department of Microbiology; Urbana, IL 61801
Grant 01-35201-09950; $195,000; 3 Years

Salmonella enterica are a large group of bacteria that are a major cause of food poisoning. A common source of human food poisoning is via *Salmonella* strains that infect farm animals. *Salmonella* strains produce distinct disease symptoms in different animals. Some *Salmonella* strains cause relatively mild disease, but strains with new , pernicious virulence properties arise rapidly. Such new strains often seem to have acquired changes in host-specificity that allowed them to infect a different animal host. If we understood what determines host specificity we could develop approaches to limit this problem, but very little is known about what determines the host-specificity of bacteria. Our experiments will use genetic approaches to compare *Salmonella* Enteritidis, which has a broad host range and infects humans, chickens, and mice, with *Salmonella* Pullorum, a host-specific strain that only infects chickens. We plan to identify the genes that determine host-specificity in these two strains of *Salmonella*. These studies will provide novel approaches to limit the development and spread of virulent *Salmonella* strains between farm animals and from farm animals to humans, and approaches to circumvent the emergence of new infectious diseases in farm animals.

2000-02457 Sources of Genetic Resistance to Reduce Fumonisin in Corn-based Foods

White, D.G.; Pataky, J.K.
University of Illinois, Urbana-Champaign; Department of Crop Sciences; Urbana, IL 61801

Grant 01-35201-10060; $175,000; 2 Years

Fumonisins are mycotoxins produced by the fungi *Fusarium verticillioides* and *F. proliferatum*. These two fungi cause Fusarium ear rot which is the most commonly occurring ear and kernel rot disease of corn. Both fungi also are often found in association with non-rotted kernels. Fumonisins cause serious health problems in animals and potentially in humans. Because of the large and diverse number of corn-based food products consumed by humans, even a minimal possibility of human health problems associated with consumption of corn products creates a serious public health concern. The long term goal of this project is to identify genetic resistance to Fusarium ear rot and to the production of fumonisins in corn that can be incorporated into widely grown commercially acceptable corn hybrids. Grain of resistant hybrids will have lower levels of fumonisins and the corn-based food products that can be made from them will also have low levels of fumonisins. In order to accomplish this goal we will evaluate 1,200 corn inbred lines, representing a large diversity of corn genotypes, in F1 crosses with a susceptible widely used, agronomically acceptable, corn inbred. Inbreds that are resistant in F1 crosses will then be studied to further identify those that can be used to create resistant, commercially acceptable, corn hybrids.

2000-02627 The Effect of Antibiotics on Shiga toxin Phage Movement in Ruminants

Acheson D.W.K.
New England Medical Center; Division of Infectious Diseases; Boston, MA 02111
Grant 01-35201-10168; $240,000; 3 Years

Shiga toxin-producing E. coli (STEC) such as *E. coli* O157:H7 are important emerging pathogens in the United States. Following intestinal infection, the production of Shiga toxins by STEC results in serious and life-threatening complications. The genes encoding these potent toxins are present on viral particles known as bacteriophages. We have previously found that when mice are infected with O157 treating them with certain antibiotics leads to an increase in toxin expression. The antibiotics also lead to increased movement of the viruses from the O157 strain into other *E. coli* in the mouse intestine. Our hypothesis is that certain antibiotics used in agriculture lead to increased movement of toxin genes carried by the viruses from one bacterial strain to another in the intestine of farm animals such as sheep and cattle. Our project will examine the effects of antibiotics used in agriculture on the movement of labeled toxin bacteriophages in the intestines of sheep. If this occurs in farm animals it is not only a critical issue that has to be considered in the use of certain antibiotics, but may go a long way in explaining the emergence of Shiga toxin positive O157 and other STEC serotypes. By understanding more about the variations between different Shiga toxin-encoding bacteriophages and learning what drives them to move from one strain to another we will be better placed to prevent the spread of bacteriophages and the potential evolution of a Shiga toxin-producing organism even more deadly than *E. coli* O157:H7.

2000-02600 Characterization of Multiple Antibiotic Resistance Among Enterohemorrhagic *Escherichia coli*

Meng, J.; White, D.G.; DebRoy, C; Zhao, S; Wagner, D.
University of Maryland, College Park; Department of Nutrition and Food Science; College Park, MD 20742
Grant 01-35201-09951; $250,000; 3 Years

During the past decade, bacteria that cause human diseases have developed resistance to many of the antibiotics commonly used for treatment. Excessive use for treating animal diseases, and subtherapeutic applications of antibiotics for disease prevention and growth promotion in animal husbandry may have played a significant role in accelerating the emergence of antibiotic-resistant bacteria. Such organisms can then be transferred from animals to humans through the food chain. Enterohemorrhagic *E. coli* (EHEC) have been a significant cause of foodborne illness in the United States. These pathogens also have been acquiring resistance phenotypes. In order to control the emergence and spread of antibiotic resistance, we need to better understand the trend of resistance and the mechanisms that lead to antibiotic resistance in foodborne pathogens. The present study is aimed at determining the progression of antimicrobial resistance phenotypes among EHEC isolates of animal and human origin over the past

thirty years. At the conclusion of the proposed research, the investigators will generate baseline data on the trend of antibiotic resistance in EHEC which may help elucidate the role that the use of antibiotics in food animals plays in the development of antibiotic resistance in foodborne pathogens. The will also have a better understanding of the genetic basis of the development of antibiotic resistance in these pathogens and of how heterologous resistance determinants are acquired and disseminated.

2000-02632 A Sensitive, Accurate and Rapid Method for Detection of Foodborne Pathogens

Kim, C.H.; Vayda, M.E.; Boettcher, K.
University of Maine, Department of Biochemistry, Microbiology, and Molecular Biology, Orono, ME 04469
Strengthening Award; Grant 01-35201-09951; $180,000; 2 Years

Our ability to ensure the safety of the nation=s food supply depends on the availability of accurate, rapid, dependable and inexpensive detection systems. The most reliable methods for assessing food contamination involve enrichment and selective culture conditions. Although sensitive enough to identify a single colony-forming unit, culture assays are time consuming, typically requiring 4 to 7 days. Over the past decade, researchers worldwide have developed several rapid diagnostic agents for common food contaminants. However, rapid and sensitive implementation of these procedures for routine food screening is hampered by the requirement for skilled operators, inhibition by food constituents, and expense. In this proposal, the investigators seek to develop an inexpensive, sensitive, non-polymerase chain reaction assay that is simple enough to be used by food processors or regulators to screen for known food contaminants. The approach employs rolling circle amplification of pathogen targets using sequence specific "molecular padlocks", which we have used for detection of other bacterial targets. We will first demonstrate the efficacy of this assay for *L. monocytogenes*, because of potential application to the dairy, blueberry, potato, meat processing and seafood industries in Maine. The long range goal of this study is to develop an automated procedure using a suite of molecular padlocks to screen for suspected food pathogens including *Salmonella spp*., *E. coli* O157:H7 and *S. aureus*.

2000-02527 Incorporating Humidity into Microbial Inactivation Models for Convection Cooking of Meats

Marks, B.P; Smith, D.M.; Ryser, E.T.
Michigan State University; Department of Agricultural Engineering; East Lansing, MI 48824-1323
Grant 01-35201-09952; $130,000; 2 Years

Major growth in the market for fully-cooked meat products and recent changes in federal regulations have resulted in a significant need to validate process lethality in commercial cooking systems. Regulations state that processors must validate new or altered process schedules by scientifically supportable means. However, existing models for pathogen inactivation, based on limited-scope laboratory tests, are insufficient to predict results in commercial convection cooking environments. Preliminary results suggest that differences in process humidity are a primary cause for this insufficiency. Consequently, the long-term goal of the proposed research is to develop improved methods for design and operation of thermal processes, based on the critical control criterion of pathogen inactivation. The specific objectives are: (1) To develop a quantitative, population-based model for thermal inactivation of a Salmonella "cocktail" in ground poultry breast meat subjected to air convection treatments, (2) To formulate novel secondary models that relate the thermal inactivation parameters to humidity, (3) To test the effect of heating rate on the validity of the inactivation model, and (4) To validate the inactivation models with pilot-scale data. Very small samples of ground meat will be inoculated with the target organisms and heated in a unique laboratory moist air heating system. Pathogen inactivation models will be developed from the resulting data. The predictive ability of these models will then be tested by comparing them to transient data from inoculated heating tests in a pilot-scale convection/steam oven. The resulting models will ultimately help optimize process design and operation to ensure product safety.

2000-02444 Genetics of Zearalenone Biosynthesis and Grain Colonization by *Gibberella zeae*

Trail, F.
Michigan State University; Department of Botany and Plant Pathology; East Lansing, MI 48824
Grant 01-35201-10062; $200,000; 3 Years

Zearalenone is a mycotoxin produced by the filamentous fungus, *Gibberella zeae*, during the colonization of grain including corn, barley and wheat. *G*. zeae, the head scab fungus, is a devastating pathogen that has caused economic losses across North Dakota, Minnesota and South Dakota that approached one billion dollars in 1993 and from $200-400 million in subsequent years. Zearalenone has estrogenic effects on mammals, including humans, and continued low levels of exposure may pose a health risk. The proposed research will focus on understanding the biosynthesis of zearalenone by *G. zeae* and the relationship between zearalenone production and colonization of stored grain by *G. zeae*. Specifically, the investigators will isolate the gene encoding the main enzyme, a polyketide synthase, involved in zearalenone production and determine the role of zearalenone in the fungal life cycle. We will isolate and sequence genes expressed during zearalenone biosynthesis and from *G. zeae* colonizing grain. Through a type of genomics analysis called microarrays, we will identify those genes expressed specifically during colonization and mycotoxin production and those expressed during both processes. These will be used to develop a model for analysis of potential control compounds using this same technology. The genomics approach will expedite analysis of gene expression during these specific stages. An understanding of zearalenone biosynthesis, its relationship to the proliferation of *G. zeae*, and the genetic mechanisms involved in grain colonization will allow practical means for control of mycotoxins produced by *G. zeae*.

2000-02524 RNA Aptamers for Food Safety Diagnostics

Miller, E.S.
North Carolina State University; Department of Microbiology; Raleigh, NC 27695-7615
Grant 01-35201-09911; $145,000; 2 Years

Using molecular selection methods, random sequences in RNA libraries can be synthesized and rare molecules selected that bind with high affinity to a chosen target molecule. The most widely used procedure, termed SELEX (*S*ystematic *E*volution of *L*igands by *EX*ponential enrichment), has generated high-affinity RNA aptamers of numerous molecules. The objectives of this program are to isolate RNA aptamers that directly bind and detect bacteria in complex mixtures such as food products, thereby eliminating elaborate culture or on-site molecular methods. Strains of *E. coli*, *Salmonella* and *Staphylococcus* will be used as whole-cell targets in isolating high-affinity RNA aptamers that bind the surface of these bacteria. The specific aims of this program are to : i) Prepare bacteria as selecting and counter-selecting targets relevant to food safety; ii) Select RNA aptamers that bind surface molecules of target bacteria with high affinity and high specificity; iii) Characterize and appropriately modify the selected diagnostic aptamers; and iv) Conjugate the RNA aptamers to assay reagents for diagnostic format. The significance of this approach is the potential to rapidly develop detection systems that function as well or better than antibodies. The system potentially can be adapted to any biological or molecule of food safety concern and employed at various stages of food processing, manufacturing or consumer delivery.

2000-02518 Safe Food Preservation

Wolf-Hall, C.E.; Garden-Robinson, J.
North Dakota State University; Departments of Cereal Science and Food and Nutrition; Fargo, ND 58104
Strengthening Award; Grant 01-35201-09953; $60,000; 2 Years

More questions are answered annually on food preservation than any other type of question in family and consumer sciences, and the North Dakota State University food preservation home page accounts for about one-third of the organization=s Internet "hits". With the proliferation of the Internet, canning and other food preservation information has become widely available, with untested recipes shared among web users and circulated on home pages. Improperly canned food could pose a public health threat due to the possibility of botulism toxin. Recently,

interest in home food preservation has grown and county extension agents have expressed concern in responding to questions about foods that have been improperly canned, particularly salsa products and canned quick breads. The long-term goal of this project is to determine the safety of home-canned food recipes and disseminate this information to consumers through consumer education directed by Extension outreach programs. The four objectives of this project are: 1) to evaluate the potentially hazardous nature of six canned quick bread recipes and six salsa recipes (as varied as possible) obtained from the Internet and popular magazines, based on pH and water activity measurement; 2) to evaluate the survival and outgrowth of *C. sporogenes* spores in two different formulations of canned quick bread recipes obtained from the Internet; 3) to develop a consumer extension publication based on the results of the research study; and 4) to develop, adapt and pilot a train-the-trainer lesson plan and media materials for extension agents detailing current safe food preservation recommendations for popular foods such as salsa.

2000-02544 Genetics and Ecology of *E. coli* O157:H7 Subpopulations

Benson, A.K.; Ju, J.
University of Nebraska; Department of Food Science and Technology; Lincoln, NE 68583-0919
Grant 01-35201-10115; $215,000; 2 Years

The presence of *E. coli* O157:H7 in cattle and the subsequent potential for transmission to humans through contaminated beef poses a significant problem to the food production industry, regulatory officials, and consumers alike. Recent studies using high resolution methods for chromosome comparison have demonstrated the existence of two genetically distinct subpopulations of this organism that have apparently unique ecological niches. Since one of the subpopulations was underrepresented among human clinical isolates that were tested, the hypothesis arose that this subpopulation is either less virulent or is inefficiently transmitted to humans through contaminated beef. In order to test this hypothesis, tools need to be developed to facilitate precise measurement of the distribution of these subpopulations and to enhance our understanding of the genetic basis for their unique ecologies. The experiments outlined in this proposal are therefore designed to use genomics and bioinformatics to identify the DNA sequence of all chromosome alterations that distinguish the two subpopulations. These sequences will provide two very critical tools to promote our understanding of the potential for these subpopulations for virulence in humans. First, a polymerase chain reaction-based method will be devised that can rapidly categorize *E. coli* O157:H7 isolates into the two different subpopulations. This method will facilitate large-scale epidemiological studies on transmission patterns of the subpopulations in animal and human environments. Secondly, understanding the nature of the chromosome alterations will provide a means for genetically dissecting the consequences of the alterations on the physiology and ecology of the subpopulations and allow us to begin making connections between selective and suppressive forces that are at work in different animal production environments.

2000-02510 Identification of *Salmonella* Adhesins for Colonization of Chickens

Wilmes-Riesenberg, M.
Bowling Green State University; Department of Biological Sciences; Bowling Green, OH 43403
Grant 01-35201-10150; $135,000; 3 Years

Salmonellosis represents a serious global problem. The incidence of infection resulting from foodborne pathogens continues to increase worldwide despite extensive research and changes at the production and processing levels. Salmonella is one of the leading causes of bacterial foodborne disease outbreaks. The long-term objective of this research is to reduce or eliminate Salmonella contamination of poultry. An understanding of the mechanism of Salmonella adherence to chicken cells could be particularly valuable in the following situations: (1) developing an efficacious live oral vaccine to prevent Salmonella colonization; (2) identifying potential changes in diet that might reduce Salmonella colonization; or (3) identifying a means of treating chicken carcasses that may reduce the numbers of attached bacteria. Preliminary data support the hypothesis that Salmonella synthesize a surface protein that is induced by growth in high iron environments and which functions in binding the bacterial pathogen to host cells. The specific goals for this grant period are: (1) to continue studies to identify Salmonella adhesins that function in

colonization of chickens; (2) to determine if any newly identified adhesin is made by other serotypes of *S. enterica* which colonize chickens; (3) to determine if induction of an immune response to these adhesins will reduce the ability of *S. typhimurium* and other Salmonella serotypes to colonize chickens; and (4) to identify genes which encode proteins regulating the iron-induced adhesin and to evaluate their role in virulence. The investigators' approach will involve using a variety of genetic and molecular biology techniques to identify mutants unable to synthesize adhesins.

2000-02621 The Role of Catabolite Repression in *Clostridium perfringens* Food Poisoning

Melville, S. B.
University of Tennessee, Memphis; Department of Microbiology and Immunology; Memphis, TN 38163
Grant 01-35201-10114; $240,000; 3 Years

Clostridium perfringens is one of the most frequent causes of food poisoning in people. *C. perfringens* often contaminates prepared food, due to its ability to produce a heat-resistant spore. After cooking, the spores germinate and the bacterium grows at an extremely rapid rate if the food is not refrigerated. When contaminated food is eaten, *C. perfringens* sporulates and produces a potent enterotoxin in the intestines, which causes the disease symptoms. An essential feature of *C. perfringens* food poisoning is the bacterium's ability to regulate the uptake and metabolism of nutrients from the food it is growing in. The global transcriptional regulatory protein, CcpA, has been shown to be a primary regulator of carbohydrate utilization in other gram positive bacteria. The three objectives of this proposal are designed to determine what role CcpA plays in *C. perfringens'* ability to grow in food products and regulate carbohydrate utilization. The objectives of the proposed research are: (1) determine if glucose acts as a catabolite repressor of alternative sugar metabolism during growth and sporulation of *C. perfringens*; (2) determine the role the CcpA regulatory protein plays in *C. perfringens*' ability to grow rapidly in foodstuffs; and (3) identify the molecular mechanism of CcpA activity in *C. perfringens*. Together, these studies will help to characterize an essential regulatory component in *C. perfringens* food poisoning. These results may also help us to come up with better food handling techniques to lower the incidence of this very common disease.

2000-02613 Verification of Safe Cooking Endpoints in Beef and Pork by Multiple Antigen ELISA

Keeton, J.T; Miller, D.R.
Texas A&M University; Department of Animal Science; College Station, TX 77843
Grant 01-35201-10169; $150,000; 2 Years

Contamination of processed meats by pathogenic organisms has caused foodborne disease outbreaks throughout the U.S. in recent years, and the publicity has provided the impetus for new procedures to reduce disease risks. Recent USDA-FSIS regulations require that pathogens be destroyed in fully-cooked products; thus, they must meet or exceed the minimum safe cooking endpoint temperatures (EPTs) and this endpoint must be verified. Current methods for determining the EPT achieved in products after cooking and in imported meats are deficient at present. Diagnostic tests based on immunoassay technology (i.e., 'dipsticks,' like those used in home pregnancy tests) have become popular in the food industry due to their reliability, ease of use, sensitivity and low cost. The investigators' studies have shown that proteins present in meat muscle tissue may be useful as post-cooking 'molecular thermometers' and they have demonstrated their potential for use in EPT tests. They also found that no single meat protein can accurately predict the EPT reached, but by determining the ratio of 3 or more proteins using a diagnostic 'dipstick' it should be possible to accurately assess the EPT reached at any time post-cooking. The investigators propose to develop a device to measure multiple meat proteins covering the appropriate EPT range(s) for beef and pork products. This concept presents a new approach and device for EPT testing. It will be rapid, accurate and inexpensive and will meet a critical need for verifying the safety of commercially cooked products, including those prepared at fast food outlets.

2000-02614 Minimizing *Salmonella* Enteritidis Invasion During Induced Molting

Ricke, S.C.

Texas A&M University, Poultry Science Department; College Station, TX 77843- 2472
Grant 01-35201-09946; $79,190; 2 Years

Salmonellosis is one of the most common foodborne diseases with an estimated 800,000 to 4 million human infections reported each year in the United States. During the past 10 to 15 years, the number of cases of gastroenteritis due to *Salmonella enterica* subspecies *enterica* serovar Enteritidis *(S.* Enteritidis*)* infections has greatly increased in the United States and Europe and by 1995, *S.* Enteritidis comprised 25% of all foodborne *Salmonella* isolates. Between 1985 and 1991, over 80% of *S.* Enteritidis infections in the United States were associated with table eggs and this may be linked to the specific stressful management practice of inducing a molt to stimulate multiple egg-laying cycles in hens. Sixty percent of the estimated 240 million laying hens nationwide are force molted with the practice growing more popular. Feed withdrawal is the primary method used in the layer industry to induce molting. However, feed withdrawal dramatically enhances *S.* Enteritidis recovery from crops, increases invasion of organs in chickens and increases horizontal transfer in flocks. The poultry industry needs alternative molting procedures that do not require feed withdrawal but allow managers to keep the economic advantages of recycling laying hens by molting without causing a *S.* Enteritidis contamination problem. The investigators plan to determine whether molt induction diets will minimize *S.* Enteritidis and if key characteristics in the chicken crop microenvironment can be linked with limiting *S.* Enteritidis colonization and pathogenesis. This will provide the poultry industry with a scientifically based rationale for possible management alternatives that reduce molting as a major risk for *S.* Enteritidis contamination.

2000-01126 LC/MS Equipment Research Enhancement for Department of Veterinary Sciences
Galey, F.
University of Wyoming; Department of Veterinary Sciences; Laramie, WY 82070
Equipment Grant; Grant 01-35201-09908; $50,000; 1 Year

Researchers in the Department of Veterinary Sciences and collaborators are currently involved in investigations about the fate of antibiotics in milk, drug disposition in minor food animal species, and the neutraceutical/natural product chemistry. The LC/MS from this proposal is a scientific instrument that will allow for detection and chemical structural identification of compounds in meat and milk. Compounds that can be detected using this instrument include antibiotics as well as other small molecules such as some natural products. Specifically, this instrument will allow for development of methods that can confirm the presence of these compounds when they are suspected of contaminating milk and meat. Once new methods are developed, the instrument will allow for development of grants to study the fate over time of a drug or natural toxin in an animal as well as studies that survey the extent of contamination of various food animal products.